DOCKET NO.: W0571.70010US02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tuschl et al.
Serial No.:	09/821,832
Confirmation No.:	6240
Filed:	March 30, 2001
For:	RNA SEQUENCE SPECIFIC MEDIATORS OF RNA INTERFERENCE
Examiner:	L. V. Wollenberger
Art Unit:	1635

Certificate of Electronic Filing Under 37 CFR 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: May 14, 2008

Sharon R. Lloyd
Sharon R. Lloyd

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 CFR §§1.56, 1.97 AND 1.98

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§1.56, 1.97 and 1.98, the Applicant requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. §1.97

This Information Disclosure Statement has been filed more than three months after the filing date of this application and after the mailing date of the first Office action, but before the mailing date of any final action under 37 C.F.R. §1.113, a Notice of Allowance under 37 C.F.R. §1.311, or an action that otherwise closes prosecution in this application.

The amount of $180.00 covering the fee set forth in 37 CFR 1.17(p) is being paid by credit card. The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith (or

with any paper hereafter filed in this application by this firm) to our Deposit Account No. 23/2825, under Docket No. W0571.70010US02.

PART II: Information Cited

The Applicant hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

The Applicant would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application

Office Actions

Serial Number	Date Mailed from PTO
10/255,568	12/31/07
11/474,738	02/13/08
11/474,930	02/13/08
11/474,919	01/08/08

The Applicant would also like to bring the following information to the Examiner's attention:

The current application is a continuation of an application filed on March 30, 2001 that claimed priority to provisional application 60/193,594 filed March 30, 2000 in the name of the same four inventors. An information disclosure statement the present applicants filed on July 7, 2005 stated:

> In the interest of insuring that the Examiner is aware of all relevant facts known to the Applications, the Examiner's attention is directed to the existence of a co-pending patent application, US Application Serial No. 10/433,050.
>
> The present application contains text at page 14 line 25 – page 15 line 13 and in example 5, which was not included in US provisional patent application 60/193,594 to which the above-identified patent application claims priority.

> The subject matter of the text at page 14 line 25 – page 15 line 13 was previously included in European Patent Application No. 00126325.0 filed in the names of Tuschl, Elbashir, and Lendeckel, to which US Application Serial No. 10/433,050 claims priority (copies enclosed). Thomas Tuschl, one of the named co-inventors of European Patent Application No. 00126325.0 and US Application Serial No. 10/433,050 is a co-inventor of the present patent application. Elbashir and Lendeckel are not named inventors on the present patent application. Applicants do not rely on the above-described text for supporting any of the currently pending claims. Additionally, the present application contains Example 5. Applicants do not rely on the above-described Example for supporting any of the currently pending claims. Rather, Applicants contend that the present claims are fully allowable without reliance upon such information. The data of Example 5 was included in US10/433,050 which claims priority to European Patent Application No. 00126325.0.

As stated, Example 5 and the information referred to as "not included in US provisional patent application 60/193,594" (cumulatively, "added information") had appeared in European Application No. 00126325.0, filed December 1, 2000, or a later application US10/433,050. Those applications had a different inventive entity from the present application, although both entities have an inventor in common (Tuschl). The present applicants have no information that the inventors in that other application other than Tuschl did not participate in developing the added information. Thus, the examiner should assume that the added information represents information developed prior to December 1, 2000 by an inventive entity different from that of the present application.

On January 24, 2006 the Patent Office in the prosecution of this application received a new declaration signed by one of the four inventors of the instant patent application, Thomas Tuschl. In the transmittal accompanying the declaration an attorney (not of record) for Dr. Tuschl states that Dr. Tuschl now believes the priority claim to European Application No. 00126325.0 is incorrect and the disclosed subject matter "regarding single strand 3' overhangs"

was derived from a different inventive entity. The other Applicants disagree with these recent statements and call them to the Examiner's attention to ensure that the Examiner is aware that a disagreement now exists among the Applicants with respect to these issues.

Dr. Tuschl is also a named inventor of US Serial Nos. 11/634,129 and 10/832,257, which are of record in this application. The inventive entity of US Serial Nos. 11/634,129 and 10/832,257 is a different inventive entity from that of this application. In arguments contained in documents filed with the Patent Office on March 3, 2008 in US Serial No. 11/634,129 and on December 6, 2006 in US Serial No. 10/832,257 an attorney for that inventive entity characterized the instant application. (copies enclosed)

If the Examiner would like any further information on any of these issues he is encouraged to contact Applicants' representative.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the Patent and Trademark Office during the examination of this application;

The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the Applicant makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the Applicant makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. §1.56(b).

By submitting this Information Disclosure Statement, the Applicant makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. §102.

Notwithstanding any statements by the Applicant, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

Respectfully submitted,

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Docket No.: W0571.70010US02
Date: May 14, 2008

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/832,257
Applicant : Thomas TUSCHL et al
Filed : April 27, 2004
TC/A.U. : 1635
Examiner : Amy Hudson Bowman

Docket No. : 2923-630
Customer No. : 6449
Confirmation No. : 4326

AMENDMENT

Director of the United States Patent
and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

December 6, 2006

Dear Sir:

In response to the Office Action of September 6, 2006, please amend the above-identified application as follows:

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 23 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-49 (Canceled).

50. (Currently amended) A method of mediating target-specific nucleic acid modifications in a mammalian cell *in vitro* comprising the steps:

contacting said mammalian cell *in vitro* with an isolated, non-enzymatically prepared, double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-23 nucleotides in length, the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated double stranded RNA molecule is substantially free from contaminants occurring in cell extracts, and

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid having a sequence portion substantially corresponding to one strand of the double-stranded RNA.

51. (Previously presented) The method according to claim 50, wherein the nucleic acid modification is RNA interference.

52. (Previously presented) The method according to claim 50, wherein said contacting comprises introducing said double-stranded RNA molecule into a target cell in which the target-specific nucleic acid modification can occur.

53. (Previously presented) The method according to claim 52, wherein said double-stranded RNA molecule is introduced into said target cell using carrier-mediated delivery or injection.

54. (Previously presented) The method according to claim 50, further comprising modulating a function of a gene in a cell *in vitro* by mediating said target-specific nucleic acid modification.

55. (Previously presented) The method according to claim 50, wherein said double stranded RNA molecule has a length from 21-23 nucleotides.

56. (Previously presented) The method according to claim 50, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 1-3 nucleotides.

57. (Previously presented) The method according to claim 56, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 2 nucleotides.

58. (Previously presented) The method according to claim 50, wherein said double stranded RNA molecule contains at least one modified nucleotide analogue.

59. (Previously presented) The method according to claim 58, wherein the modified nucleotide analogue is selected from sugar- or backbone modified ribonucleotides.

60. (Previously presented) The method according to claim 59, wherein the nucleotide analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, $N(R)_2$ and CN, and wherein R is selected from the group consisting of C_1-C_6 alkyl, alkenyl and alkynyl, and halo is selected from the group consisting of F, Cl, Br and I.

61. (Previously presented) The method according to claim 59, wherein the nucleotide analogue is a backbone-modified ribonucleotide containing a phosphorothioate group.

62. (Currently amended) A method for determining the function of a gene in a <u>mammalian</u> cell *in vitro*, comprising

contacting said <u>mammalian</u> cell *in vitro* with an isolated<u>, non-enzymatically prepared</u> double-stranded RNA molecule under conditions wherein

target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-23 nucleotides in length, and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 5 nucleotides at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated double stranded RNA molecule is substantially free from contaminants occurring in cell extracts,

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid which is part of a gene, wherein said target nucleic acid has a sequence portion substantially corresponding to the double-stranded RNA, and

determining the function of said gene based on the results of said modification.

63. (Previously presented) The method according to claim 62, wherein the gene is associated with a pathological condition.

64. (Previously presented) The method according to claim 63, wherein the gene is a pathogen-associated gene.

65. (Previously presented) The method according to claim 64, wherein the gene is a viral gene.

66. (Previously presented) The method according to claim 63, wherein the gene is a tumor-associated gene.

67. (Previously presented) The method according to claim 63, wherein the gene is an autoimmune disease-associated gene.

68. (Currently amended) A method for mediating cleavage of a target mRNA in a mammalian cell *in vitro* comprising contacting a mammalian cell or organism with an isolated, non-enzymatically ~~synthetically~~ prepared double stranded RNA molecule consisting of 19-23 nucleotides in length under conditions wherein cleavage of a target mRNA can occur, wherein said ~~synthetically~~ non-enzymatically prepared double stranded RNA has a double stranded region of 16-22 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule and wherein one strand of said double stranded RNA is complementary to said target mRNA, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

69. (Currently amended) An improved method for mediating the cleavage of a target RNA in a mammalian cell *in vitro*, the improvement comprising ~~the use of~~ cleaving a target RNA with an isolated, ~~synthetically~~ non-enzymatically prepared double stranded ~~region~~ RNA consisting of 19-23 nucleotides in length, wherein said

double stranded RNA has a double stranded region of 16-22 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded RNA molecule, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

70. (Currently amended) A method of mediating target-specific nucleic acid modifications in a mammalian cell *in vitro* comprising the steps:

contacting said mammalian cell *in vitro* with an isolated, non-enzymatically prepared double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-25 nucleotides in length, the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts, and

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid having a sequence portion substantially corresponding to the double-stranded RNA.

71. (Previously presented) The method according to claim 70, wherein the nucleic acid modification is RNA interference.

72. (Previously presented) The method according to claim 70, wherein said contacting comprises introducing said double-stranded RNA molecule into a target cell in which the target-specific nucleic acid modification can occur.

73. (Previously presented) The method according to claim 72, wherein said double-stranded RNA molecule is introduced into said target cell using carrier-mediated delivery or injection.

74. (Previously presented) The method according to claim 70, further comprising modulating a function of a gene in a cell *in vitro* by mediating said target-specific nucleic acid modification.

75. (Previously presented) The method according to claim 70, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 1-3 nucleotides.

76. (Previously presented) The method according to claim 75, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 2 nucleotides.

77. (Previously presented) The method according to claim 70, wherein said double stranded RNA molecule contains at least one modified nucleotide analogue.

78. (Previously presented) The method according to claim 77, wherein the modified nucleotide analogue is selected from sugar- or backbone modified ribonucleotides.

79. (Previously presented) The method according to claim 78, wherein the nucleotide analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, $N(R)_2$ and CN, and wherein R is selected from the group consisting of C_1-C_6 alkyl, alkenyl and alkynyl, and halo is selected from the group consisting of F, Cl, Br and I.

80. (Previously presented) The method according to claim 78, wherein the nucleotide analogue is a backbone-modified ribonucleotide containing a phosphorothioate group.

81. (Currently amended) A method for determining the function of a gene in a <u>mammalian</u> cell *in vitro*, comprising

contacting said <u>mammalian</u> cell *in vitro* with an isolated<u>, non-enzymatically prepared</u> double-stranded RNA molecule under conditions wherein

target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-25 nucleotides in length, and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts,

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid which is part of a gene, wherein said target nucleic acid has a sequence portion substantially corresponding to the double-stranded RNA, and

determining the function of said gene based on the results of said modification.

82. (Previously presented) The method according to claim 81, wherein the gene is associated with a pathological condition.

83. (Previously presented) The method according to claim 82, wherein the gene is a pathogen-associated gene.

84. (Previously presented) The method according to claim 82, wherein the gene is a viral gene.

85. (Previously presented) The method according to claim 82, wherein the gene is a tumor-associated gene.

86. (Previously presented) The method according to claim 82, wherein the gene is an autoimmune disease-associated gene.

87. (Currently amended) A method for mediating cleavage of a target mRNA in a mammalian cell *in vitro* comprising contacting a cell or organism with an isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule consisting of 19-25 nucleotides in length under conditions wherein cleavage of a target mRNA can occur, wherein said ~~synthetically~~ non-enzymatically prepared double stranded RNA has a double stranded region of 16-24 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule and wherein one strand of said double stranded RNA is complementary to said target mRNA, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

88. (Currently amended) An improved method for mediating the cleavage of a target RNA in a mammalian cell *in vitro*, the improvement comprising ~~the use of~~ cleaving a target RNA with an isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule consisting of 19-25 nucleotides in length, wherein

said double stranded RNA molecule has a double stranded region of 16-24 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

89. (Currently amended) A method of mediating target-specific nucleic acid modifications in a mammalian cell *in vitro* comprising the steps:

contacting said mammalian cell *in vitro* with an isolated, non-enzymatically prepared double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-23 nucleotides in length and consists of a single double stranded region and one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts, and

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid having a sequence portion substantially corresponding to one strand of the double-stranded RNA.

90. (Previously presented) The method according to claim 89, wherein the nucleic acid modification is RNA interference.

91. (Previously presented) The method according to claim 89, wherein said contacting comprises introducing said double-stranded RNA molecule into a target cell in which the target-specific nucleic acid modification can occur.

92. (Previously presented) The method according to claim 91, wherein said double-stranded RNA molecule is introduced into said target cell using carrier-mediated delivery or injection.

93. (Currently amended) The method according to claim 89, further comprising modulating a function of a gene in a <u>mammalian</u> cell *in vitro* by mediating said target-specific nucleic acid modification.

94. (Previously presented) The method according to claim 89, wherein said double stranded RNA molecule has a length from 21-23 nucleotides.

95. (Previously presented) The method according to claim 89, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 1-3 nucleotides.

96. (Previously presented) The method according to claim 95, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 2 nucleotides.

97. (Previously presented) The method according to claim 89, wherein said double stranded RNA molecule contains at least one modified nucleotide analogue.

98. (Previously presented) The method according to claim 97, wherein the modified nucleotide analogue is selected from sugar- or backbone modified ribonucleotides.

99. (Previously presented) The method according to claim 98, wherein the nucleotide analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, $N(R)_2$ and CN, and wherein R is selected from the group consisting of C_1-C_6 alkyl, alkenyl and alkynyl, and halo is selected from the group consisting of F, Cl, Br and I.

100. (Previously presented) The method according to claim 98, wherein the nucleotide analogue is a backbone-modified ribonucleotide containing a phosphorothioate group.

101. (Currently amended) A method for determining the function of a gene in a <u>mammalian</u> cell *in vitro*, comprising

contacting said mammalian cell *in vitro* with an isolated, non-enzymatically prepared double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-23 nucleotides in length, and consists of a single double stranded region and one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts,

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid which is part of a gene, wherein said target nucleic acid has a sequence portion substantially corresponding to one strand of the double-stranded RNA, and

determining the function of said gene based on the results of said modification.

102. (Previously presented) The method according to claim 101, wherein the gene is associated with a pathological condition.

103. (Previously presented) The method according to claim 102, wherein the gene is a pathogen-associated gene.

104. (Previously presented) The method according to claim 103, wherein the gene is a viral gene.

105. (Previously presented) The method according to claim 103, wherein the gene is a tumor-associated gene.

106. (Previously presented) The method according to claim 103, wherein the gene is an autoimmune disease-associated gene.

107. (Currently amended) A method for mediating cleavage of a target mRNA in a mammalian cell *in vitro* comprising contacting a mammalian cell or organism with an isolated, non-enzymatically ~~synthetically~~ prepared double stranded RNA molecule consisting of 19-23 nucleotides in length under conditions wherein cleavage of a target mRNA can occur, wherein said ~~synthetically~~ non-enzymatically prepared double stranded RNA consists of a single double stranded region of 16-22 nucleotides in length and one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule and wherein one strand of said double stranded RNA is complementary to said target mRNA, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

108. (Currently amended) An improved method for mediating the cleavage of a target RNA in a mammalian cell *in vitro*, the improvement comprising ~~the use of~~ cleaving a target RNA with an isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA consisting of 19-23 nucleotides in length, wherein said double

stranded RNA consists of a single double stranded region of 16-22 nucleotides in length and one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, ~~synthetically~~ <u>non-enzymatically</u> prepared double stranded RNA is substantially free from contaminants occurring in cell extracts.

109. (Currently amended) A method of mediating target-specific nucleic acid modifications in a <u>mammalian</u> cell *in vitro* comprising the steps:

contacting said <u>mammalian</u> cell *in vitro* with an isolated<u>, non-enzymatically prepared</u> double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-25 nucleotides in length, and consists of a single double stranded region and one or two single stranded regions of 1 to 5 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts and

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid having a sequence portion substantially corresponding to one strand of the double-stranded RNA.

110. (Previously presented) The method according to claim 109, wherein the nucleic acid modification is RNA interference.

111. (Previously presented) The method according to claim 109, wherein said contacting comprises introducing said double-stranded RNA molecule into a target cell in which the target-specific nucleic acid modification can occur.

112. (Previously presented) The method according to claim 111, wherein said double-stranded RNA molecule is introduced into said target cell using carrier-mediated delivery or injection.

113. (Previously presented) The method according to claim 109, further comprising modulating a function of a gene in a cell *in vitro* by mediating said target-specific nucleic acid modification.

114. (Previously presented) The method according to claim 109, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 1-3 nucleotides.

115. (Previously presented) The method according to claim 114, wherein at least one strand of said double stranded RNA molecule has a 3'-overhang of 2 nucleotides.

116. (Previously presented) The method according to claim 109, wherein said double stranded RNA molecule contains at least one modified nucleotide analogue.

117. (Previously presented) The method according to claim 116, wherein the modified nucleotide analogue is selected from sugar- or backbone modified ribonucleotides.

118. (Previously presented) The method according to claim 117, wherein the nucleotide analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, $N(R)_2$ and CN, and wherein R is selected from the group consisting of C_1-C_6 alkyl, alkenyl and alkynyl, and halo is selected from the group consisting of F, Cl, Br and I.

119. (Previously presented) The method according to claim 117, wherein the nucleotide analogue is a backbone-modified ribonucleotide containing a phosphorothioate group.

120. (Currently amended) A method for determining the function of a gene in a mammalian cell *in vitro*, comprising

contacting said mammalian cell *in vitro* with an isolated, non-enzymatically prepared double-stranded RNA molecule under conditions wherein target-specific nucleic acid modifications can occur, wherein said double stranded RNA molecule consists of 19-25 nucleotides in length, and consists of a single double stranded region and one or two single stranded regions of 1 to 5 nucleotides

each at the 3' ends of the strands of said double-stranded molecule, and wherein said isolated, double stranded RNA molecule is substantially free from contaminants occurring in cell extracts,

mediating a target-specific nucleic acid modification effected by the double-stranded RNA towards a target nucleic acid ~~which is part of a gene~~, wherein said target nucleic acid has a sequence portion substantially corresponding to one strand of the double-stranded RNA, and

determining the function of said gene based on the results of said modification.

121. (Previously presented) The method according to claim 120, wherein the gene is associated with a pathological condition.

122. (Previously presented) The method according to claim 121, wherein the gene is a pathogen-associated gene.

123. (Previously presented) The method according to claim 121, wherein the gene is a viral gene.

124. (Previously presented) The method according to claim 121, wherein the gene is a tumor-associated gene.

125. (Previously presented) The method according to claim 121, wherein the gene is an autoimmune disease-associated gene.

126. (Currently amended) A method for mediating cleavage of a target mRNA in a mammalian cell *in vitro* comprising contacting a mammalian cell or organism with an isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule consisting of 19-25 nucleotides in length under conditions wherein cleavage of a target mRNA can occur, wherein said ~~synthetically~~ non-enzymatically prepared double stranded RNA consists of a single double stranded region of 16-24 nucleotides in length and one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule and wherein one strand of said double stranded RNA is complementary to said target mRNA, and wherein said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

127. (Currently amended) An improved method for mediating the cleavage of a target RNA in a mammalian cell *in vitro*, the improvement comprising ~~the use of~~ cleaving a target RNA with an isolated, non-enzymatically ~~synthetically~~ prepared double stranded ~~region~~ RNA consisting of 19-25 nucleotides in length, wherein said double stranded RNA consists of a single double stranded region of 16-24 nucleotides in length and one or two single stranded regions of 1 to 3 nucleotides each at the 3' ends of the strands of said double-stranded molecule, and wherein

said isolated, ~~synthetically~~ non-enzymatically prepared double stranded RNA molecule is substantially free from contaminants occurring in cell extracts.

REMARKS:

In the Office Action dated September 6, 2006, claims 50-127 in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 50-127 remain in this application and claims 1-49 have been canceled.

Applicants thank Examiners Bowman and Schultz for the interview on November 14, 2006. Proposed amendments for overcoming the rejections were discussed. Support for the amendments regarding mammalian cells can be found on page 3, lines 6-9 and in example 2 of the present application. Support for the amendments regarding non-enzymatically prepared double stranded RNA molecules can be found on page 7, lines 14-16 of the present application.

Claims 69, 88, 108 and 127 were rejected under 35 USC §112, second paragraph, as indefinite and under 35 USC §101. The claims have been amended to delete the term "use" and to recite "the improvement comprising cleaving a target RNA with...". In view of these amendments applicants request that these rejections be withdrawn.

Claims 69 and 127 were rejected under 35 USC §112, second paragraph, as indefinite. Claims 69 and 127 were amended to indicate that the isolated, non-enzymatically prepared double stranded RNA consists of 19-23 nucleotides in length, wherein said double stranded RNA has a double stranded region of 16-22 nucleotides in length. In view of these amendments, applicants request that these rejections be withdrawn.

Claim 120 was rejected under 35 USC §112, second paragraph, as indefinite. During the November 14, 2006 interview, the steps of "mediating" and "determining" were discussed as active steps. In view of the fact that "mediating" and "determining" were determined to be active steps, applicants request that this rejection be withdrawn.

Claim 50 was rejected under 35 USC §112, first paragraph, as lacking an adequate written description. Claim 50 has been amended as suggested in the office action. In view of this amendment, applicants request that this rejection be withdrawn.

Claims 50-57, 62, 68, 70-76, 81, 87-96, 101, 107, 109-115, 120, and 126 were rejected under 35 USC §102(b) as anticipated by Zamore as evidenced by Zhang. Zamore used a Drosophila system and does not disclose or suggest that a mammalian system can be used. Zhang suggests only the enzymatic cleavage of long double stranded RNAs. Therefore, Zamore as evidenced by Zhang does not anticipate the presently claimed method for mediating target specific nucleic acid modifications in a mammalian cell by contacting a cell with an isolated, non-enzymatically prepared double stranded RNA molecule and applicants request that this rejection be withdrawn.

Claims 50-68, 70-87, 89-107 and 109-126 were rejected under 35 USC §103 (a) as unpatentable over Zamore as evidenced by Zhang, in view of Parrish and Fire. As discussed above, Zamore as evidenced by Zhang does not suggest or disclose the presently claimed method for mediating target specific nucleic acid modifications in a mammalian cell by contacting a cell with an isolated, non-enzymatically prepared double stranded RNA molecule. Parrish and Fire do not cure this deficiency as Parrish and Fire

both disclose long molecules which may inherently be enzymatically cleaved by Dicer as disclosed by Zhang. Applicants also point out that both Parrish and Fire studied *C. elegans*. The present inventors were the first to demonstrate siRNA-mediated gene silencing in mammalian cells. In view of the above amendments and discussion, applicants request that this rejection be withdrawn.

During the prosecution of co-pending application serial no. 10/255,568, a provisional double patenting rejection was made over the present application. Applicants point out that these applications have different inventive entities, are not commonly assigned and were not made under a joint research agreement. In addition, the present claims require a single stranded region of 1 to 5 nucleotides at the 3' end of at least one of the strands of the double stranded molecule. The single stranded regions would not have been obvious over the general disclosure in application serial no. 10/255,568. The present claims also indicate that the cells are mammalian cells. Application serial no. 10/255,568 now improperly includes the mammalian cell data generated by the present inventors and now improperly claims priority back to EP 00126325.0. In the absence of the data from the present inventors, application serial no. 10/255,568 does not provide an enabling disclosure of a method for mediating target specific nucleic acid modifications in a mammalian cell. As indicated in the IDS filed on July 11, 2005 in application serial no. 09/821,832 (the parent application of application serial no. 10/255,568), the subject matter of the text at page 14, line 25 to page 15 line 13 of application serial no. 09/821,832 was disclosed in EP 00126325.0 which lists Tuschl, Elbashir and Lendeckel as inventors. This subject matter relates to the single stranded overhangs and the example regarding mammalian cells. Tuschl, Elbashir and Lendeckel are the inventors listed for the present

application and are different from the inventors of application serial no. 10/255,568. In view of the above discussion, applicants contend that a double patenting rejection over co-pending application serial no. 10/255,568 would be improper for the present application.

Applicants respectfully submit that all of claims 50-127 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

By

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MCK/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 11/634,129
Applicant : Thomas Tuschl et al.
Filed : December 6, 2006
TC/A.U. : 1635
Examiner : Amy Hudson Bowman

Docket No. : 2923-793
Customer No. : 6449
Confirmation No. : 4466

RESPONSE TO NOTICE OF NON-COMPLIANT AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

February 28, 2008

Sir:

In response to the Notice of Non-Compliant Amendment dated February 25, 2008, enclosed is a corrected Response.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

By

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MCK/cb

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	: 11/634,129
Applicant	: Thomas Tuschl et al.
Filed	: December 6, 2006
TC/A.U.	: 1635
Examiner	: Amy Hudson Bowman

Docket No. : 2923-793
Customer No. : 6449
Confirmation No. : 4466

RESPONSE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

January 22, 2008

Sir:

In response to the Office Action of September 19, 2007, please amend the application as follows.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 11 of this paper.

Amendments to the Drawings begin on page 17 of this paper.

Remarks/Arguments begin on page 19 of this paper.

Substitute drawings are attached following page 22 of this paper.

Amendments to the Specification

Please replace the paragraph which spans page 19 lines 24-32 through page 20 lines 1-10 with the following amended paragraph:

Figure 11 Parts I - III: Variation of the 3' overhang of duplexes of 21-nt siRNAs. [Part I (A)] Outline of the experimental strategy. The capped and polyadenylated sense target mRNA is depicted and the relative positions of sense and antisense siRNAs are shown. Eight series of duplexes, according to the eight different antisense strands were prepared. The siRNA sequences and the number of overhanging nucleotides were changed in 1-nt steps. [Part I (B)] Normalized relative luminescence of target luciferase (Photinus pyralis, Pp-luc) to control luciferase (Renilla reniformis, Rr-luc) in D. melanogaster embryo lysate in the presence of 5 nM blunt-ended dsRNAs. The luminescence ratios determined in the presence of dsRNA were normalized to the ratio obtained for a buffer control (bu, black bar). Normalized ratios less than 1 indicate specific interference. ~~(C-J)~~ [Part I (C-D), Part II (E-G), Part III (H-J)] Normalized interference ratios for eight series of 21-nt siRNA duplexes. The sequences of siRNA duplexes are depicted above the bar graphs. Each panel shows the interference ratio for a set of duplexes formed with a given antisense guide siRNA and 5 different sense siRNAs. The number of overhanging nucleotides (3' overhang, positive numbers; 5' overhangs, negative numbers) is indicated on the x-axis. Data points were averaged from at least 3 independent experiments, error bars represent standard deviations.

Please replace the paragraph on page 20 lines 12-18 with the following amended paragraph:

Figure 12: Variation of the length of the sense strand of siRNA duplexes. [Part I (A)] Graphic representation of the experiment. Three 21-nt antisense strands were paired with eight sense siRNAs. The siRNAs were changed in length at their 3' end. The 3' overhang of the antisense siRNA was 1-nt [Part I (B)], 2-nt [Part II (C)], or 3-nt [Part II (D)] while the sense siRNA overhang was varied for each series.

The sequences of the siRNA duplexes and the corresponding interference ratios are indicated.

Please replace the paragraph on page 20 lines 20-26 with the following amended paragraph:

Figure 13: Variation of the length of siRNA duplexes with preserved 2-nt 3' overhangs.
(A) Graphic representation of the experiment. The 21-nt siRNA duplex is identical in sequence to the one shown in Figure 11 Part III H or 12 Part II C. The siRNA duplexes were extended to the 3' side of the sense siRNA (B) or the 5' side of the sense siRNA (C). The siRNA duplex sequences and the respective interference ratios are indicated.

Please replace the paragraph on page 22 lines 1-6 with the following amended paragraph:

Figure 17: Sequence variation of the 3' overhang of siRNA duplexes.
The 2-nt 3' overhang (NN, in gray) was changed in sequence and composition as indicated (T, 2'-deoxythymidine, dG, 2'-deoxyguanosine; asterisk, wild-type siRNA duplex). Normalized interference ratios were determined as described in Figure 11 Parts I-III. The wild-type sequence is the same as depicted in Figure 14.

Please replace the paragraph on page 22 lines 8-14 with the following amended paragraph:

Figure 18: Sequence specificity of target recognition.
The sequences of the mismatched siRNA duplexes are shown, modified sequence segments or single nucleotides are underlayed in gray. The reference duplex (ref) and the siRNA duplexes 1 to 7 contain 2'-deoxythymidine 2-nt overhangs. The silencing efficiency of the thymidine-modified reference duplex was comparable to the wild-type

sequence (Figure 17). Normalized interference ratios were determined as described in Figure 11 Parts I-III.

Please replace the paragraph which spans page 23 lines 10-31 through page 24 lines 1-2 with the following amended paragraph:

1.1.2RNA Synthesis

Standard procedures were used for in vitro transcription of RNA from PCR templates carrying T7 or SP6 promoter sequences, see for example (Tuschl et al., 1998). Synthetic RNA was prepared using Expedite RNA phosphoramidites (Proligo). The 3' adapter oligonucleotide was synthesized using dimethoxytrityl-1,4-benzenedimethanol-succinyl-aminopropyl-CPG. The oligoribonucleotides were deprotected in 3 ml of 32% ammonia/ethanol (3/1) for 4 h at 55°C (Expedite RNA) or 16 h at 55°C (3' and 5' adapter DNA/RNA chimeric oligonucleotides) and then desilylated and gel-purified as described previously (Tuschl et al., 1993). RNA transcripts for dsRNA preparation including long 3' overhangs were generated from PCR templates that contained a T7 promoter in sense and an SP6 promoter in antisense direction. The transcription template for sense and antisense target RNA was PCR-amplified with ~~GCGTAATACGACTCACTATAGAACAATTGCTTTTACAG~~ GCG**TAATACGACTCACTATA**GAACAATTGCTTTTACAG (~~underlined~~ bold, T7 promoter) [SEQ ID NO: 1] as 5' primer and ~~ATTTAGGTGACACTATAGGCATAAAGAATTGAAGA~~ **ATTTAGGTGACACTATA**GGCATAAAGAATTGAAGA (~~underlined~~ bold, SP6 promoter) [SEQ ID NO:2] as 3' primer and the linearized Pp-luc plasmid (pGEM-luc sequence) (Tuschl et al., 1999) as template; the T7-transcribed sense RNA was 177 nt long with the Pp-luc sequence between pos. 113-273 relative to the start codon and followed by 17 nt of the complement of the SP6 promoter sequence at the 3' end. Transcripts for blunt-ended dsRNA formation were prepared by transcription from two different PCR products which only contained a single promoter sequence.

Please replace the paragraph which spans page 25 lines 4-32 through page 26 lines 1-15 with the following amended paragraph:

1.1.4Cloning of ~21 nt RNAs

The 21 nt RNAs were produced by incubation of radiolabeled dsRNA in Drosophila lysate in absence of target RNA (200 Fl reaction, 1 h incubation, 50 nM dsP111, or 100 nM dsP52 or dsP39). The reaction mixture was subsequently treated with proteinase K (Tuschl et al., 1999) and the dsRNA-processing products were separated on a denaturing 15% polyacrylamide gel. A band, including a size range of at least 18 to 24 nt, was excised, eluted into 0.3 M NaCl overnight at 4°C and in siliconized tubes. The RNA was recovered by ethanol-precipitation and dephosphorylated (30 Fl reaction, 30 min, 50°C, 10 U alkaline phosphatase, Roche). The reaction was stopped by phenol/chloroform extraction and the RNA was ethanol-precipitated. The 3' adapter oligonucleotide (pUUUaaccgcatccttctcx: uppercase, RNA; lowercase, DNA; p, phosphate; x, 4-hydroxymethylbenzyl) [SEQ ID NO: 100] was then ligated to the dephosphorylated ~21 nt RNA (20 Fl reaction, 30 min, 37°C, 5 FM 3' adapter, 50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 0.2 mM ATP, 0.1 mg/ml acetylated BSA, 15% DMSO, 25 U T4 RNA ligase, Amersham-Pharmacia) (Pan and Uhlenbeck, 1992). The ligation reaction was stopped by the addition of an equal volume of 8 M urea/50 mM EDTA stopmix and directly loaded on a 15% gel. Ligation yields were greater 50%. The ligation product was recovered from the gel and 5'-phosphorylated (20 Fl reaction, 30 min, 37°C, 2 mM ATP, 5 U T4 polynucleotide kinase, NEB). The phosphorylation reaction was stopped by phenol/chloroform extraction and RNA was recovered by ethanol-precipitation. Next, the 5' adapter (tactaatacgactcactAAA: uppercase, RNA; lowercase, DNA) [SEQ ID NO: 101] was ligated to the phosphorylated ligation product as described above. The new ligation product was gel-purified and eluted from the gel slice in the presence of reverse transcription primer (GACTAGCTG**GAATTC**AAGGATGCGGTTAAA: bold, Eco RI site) [SEQ ID NO: 3] used as carrier. Reverse transcription (15 Fl reaction, 30 min, 42°C, 150 U Superscript

II reverse transcriptase, Life Technologies) was followed by PCR using as 5' primer CAGCCAACG**GAATTC**ATACGACTCACTAAA (bold, Eco RI site) [SEQ ID NO: 4] and the 3' RT primer. The PCR product was purified by phenol/ chloroform extraction and ethanol-precipitated. The PCR product was then digested with Eco RI (NEB) and concatamerized using T4 DNA ligase (high conc., NEB). Concatamers of a size range of 200 to 800 bp were separated on a low-melt agarose gel, recovered from the gel by a standard melting and phenol extraction procedure, and ethanol-precipitated. The unpaired ends were filled in by incubation with Taq polymerase under standard conditions for 15 min at 72°C and the DNA product was directly ligated into the pCR2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen). Colonies were screened using PCR and M13-20 and M13 Reverse sequencing primers. PCR products were directly submitted for custom sequencing (Sequence Laboratories Göttingen GmbH, Germany). On average, four to five 21mer sequences were obtained per clone.

Please replace the paragraph on page 43 lines 1-22 with the following amended paragraph:

For mapping of sense target RNA cleavage, a 177-nt transcript was generated, corresponding to the firefly luciferase sequence between positions 113-273 relative to the start codon, followed by the 17-nt complement of the SP6 promoter sequence. For mapping of antisense target RNA cleavage, a 166-nt transcript was produced from a template, which was amplified from plasmid sequence by PCR using 5' primer ~~TAATACGACTCACTATAGAGCCCATATCGTTTCATA~~ **TAATACGACTCACTATAGA**GCCCATATCGTTTCATA (T7 promoter ~~underlined~~ in bold) [SEQ ID NO: 5] and 3' primer AGAGGATGGAACCGCTGG [SEQ ID NO: 6]. The target sequence corresponds to the complement of the firefly luciferase sequence between positions 50-215 relative to the start codon. Guanylyl transferase labelling was performed as previously described (Zamore et al., 2000). For mapping of target RNA cleavage, 100 nM siRNA duplex was incubated with 5 to 10 nM target RNA in D.

melanogaster embryo lysate under standard conditions (Zamore et al., 2000) for 2 h at 25EC. The reaction was stopped by the addition of 8 volumes of proteinase K buffer (200 mM Tris-HCl pH 7.5, 25 mM EDTA, 300 mM NaCl, 2% w/v sodium dodecyl sulfate). Proteinase K (E.M. Merck, dissolved in water) was added to a final concentration of 0.6 mg/ml. The reactions were then incubated for 15 min at 65EC, extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with 3 volumes of ethanol. Samples were located on 6% sequencing gels. Length standards were generated by partial RNase T1 digestion and partial base hydrolysis of the cap-labelled sense or antisense target RNAs.

Please replace the paragraph which spans page 43 lines 26-32 through page 44 lines 1-15 with the following amended paragraph:

3.2.1 Variation of the 3' overhang in duplexes of 21-nt siRNAs

As described above, 2 or 3 unpaired nucleotides at the 3' end of siRNA duplexes were more efficient in target RNA degradation than the respective blunt-ended duplexes. To perform a more comprehensive analysis of the function of the terminal nucleotides, we synthesized five 21-nt sense siRNAs, each displayed by one nucleotide relative to the target RNA, and eight 21-nt antisense siRNAs, each displaced by one nucleotide relative to the target (Figure 11 Part I A). By combining sense and antisense siRNAs, eight series of siRNA duplexes with synthetic overhanging ends were generated covering a range of 7-nt 3' overhang to 4-nt 5' overhang. The interference of siRNA duplexes was measured using the dual luciferase assay system (Tuschl et al., 1999; Zamore et al., 2000). siRNA duplexes were directed against firefly luciferase mRNA, and sea pansy luciferase mRNA was used as internal control. The luminescence ratio of target to control luciferase activity was determined in the presence of siRNA duplex and was normalized to the ratio observed in the absence of dsRNA. For comparison, the interference ratios of long dsRNAs (39 to 504 pb) are shown in Figure 11 Part I B. The interference ratios were determined at concentrations of 5 nM for long dsRNAs (Figure 11 Part I A) and at 100 nM for siRNA duplexes (Figure 11~~C-J~~ Part I C-D, Part II

E-G, Part III -J). The 100 nM concentrations of siRNAs was chosen, because complete processing of 5 nM 504 bp dsRNA would result in 120 nM total siRNA duplexes.

Please replace the paragraph on page 44 lines 17-32 with the following amended paragraph:

The ability of 21-nt siRNA duplexes to mediate RNAi is dependent on the number of overhanging nucleotides or base pairs formed. Duplexes with four to six 3' overhanging nucleotides were unable to mediate RNAi (Figure 11~~C-F~~ Part I C-D, Part II E-F), as were duplexes with two or more 5' overhanging nucleotides (Figure 11~~G-J~~ Part II G, Part III H-J). The duplexes with 2-nt 3' overhangs were most efficient in mediating RNA interference, though the efficiency of silencing was also sequence-dependent, and up to 12-fold differences were observed for different siRNA duplexes with 2-nt 3' overhangs (compare Figure 11 ~~D-H~~ Part I D, Part II E-G, Part III H). Duplexes with blunted ends, 1-nt 5' overhang or 1- to 3-nt 3' overhangs were sometimes functional. The small silencing effect observed for the siRNA duplex with 7-nt 3' overhang (Figure 11 Part I C) may be due to an antisense effect of the long 3' overhang rather than due to RNAi. Comparison of the efficiency of RNAi between long dsRNAs (Fig. 11 Part I B) and the most effective 21-nt siRNA duplexes (Fig. 11 ~~E, G, H~~ Part II E, Part II G, Part III H) indicates that a single siRNA duplex at 100 nM concentration can be as effective as 5 nM 504 bp dsRNA.

Please replace the paragraph on page 45 lines 1-22 with the following amended paragraph:

3.2.2 Length variation of the sense siRNA paired to an invariant 21-nt antisense siRNA

In order to investigate the effect of length of siRNA on RNAi, we prepared 3 series of siRNA duplexes, combining three 21-nt antisense strands with eight, 18- to 25-nt sense strands. The 3' overhang of the antisense siRNA was fixed to 1, 2, or 3 nt in

each siRNA duplex series, while the sense siRNA was varied at its 3' end (Figure 12 Part I A). Independent of the length of the sense siRNA, we found that duplexes with 2-nt 3' overhang of antisense siRNA (Figure 12 Part II C) were more active than those with 1- or 3-nt 3' overhang (Figure 12~~B, D~~ Part I B, Part II D). In the first series, with 1-nt 3' overhang of antisense siRNA, duplexes with a 21- and 22-nt sense siRNAs, carrying a 1- and 2-nt 3' overhang of sense siRNA, respectively, were most active. Duplexes with 19- to 25-nt sense siRNAs were also able to mediate RNA, but to a lesser extent. Similarly, in the second series, with 2-nt overhang of antisense siRNA, the 21-nt siRNA duplex with 2-nt 3' overhang was most active, and any other combination with the 18- to 25-nt sense siRNAs was active to a significant degree. In the last series, with 3-nt antisense siRNA 3' overhang, only the duplex with a 20-nt sense siRNA and the 2-nt sense 3' overhang was able to reduce target RNA expression. Together, these results indicate that the length of the siRNA as well as the length of the 3' overhang are important, and that duplexes of 21-nt siRNAs with 2-nt 3' overhang are optimal for RNAi.

Please replace the paragraph which spans page 45 lines 24-32 through page 46 lines 1-3 with the following amended paragraph:

3.2.3 Length variation of siRNA duplexes with a constant 2-nt 3' overhang

We then examined the effect of simultaneously changing the length of both siRNA strands by maintaining symmetric 2-nt 3' overhangs (Figure 13A). Two series of siRNA duplexes were prepared including the 21-nt siRNA duplex of Figure 11 Part III H as reference. The length of the duplexes was varied between 20 to 25 bp by extending the base-paired segment at the 3' end of the sense siRNA (Figure 13B) or at the 3' end of the antisense siRNA (Figure 13C). Duplexes of 20 to 23 bp caused specific repression of target luciferase activity, but the 21-nt siRNA duplex was at least 8-fold more efficient than any of the other duplexes. 24- and 25-nt siRNA duplexes did not result in any detectable interference. Sequence-specific effects were minor as variations on both ends of the duplex produced similar effects.

Please replace the paragraph which spans page 46 lines 16-31 through page 47 lines 1-2 with the following amended paragraph:

3.2.5 Definition of target RNA cleavage sites

Target RNA cleavage positions were previously determined for 22-nt siRNA duplexes and for a 21-nt/22-nt duplex. It was found that the position of the target RNA cleavage was located in the centre of the region covered by the siRNA duplex, 11 or 12 nt downstream of the first nucleotide that was complementary to the 21- or 22-nt siRNA guide sequence. Five distinct 21-nt siRNA duplexes with 2-nt 3' overhang (Figure 15A) were incubated with 5' cap-labelled sense or antisense target RNA in D. melanogaster lysate (Tuschl et al., 1999; Zamore et al., 2000). The 5' cleavage products were resolved on sequencing gels (Figure 15B). The amount of sense target RNA cleaved correlates with the efficiency of siRNA duplexes determined in the translation-based assay, and siRNA duplexes 1, 2 and 4 (Figure 15B and 11~~H, G, E~~ Part II E, Part II G, Part III H) cleave target RNA faster than duplexes 3 and 5 (Figure 15B and 11~~F, D~~ Part I D, Part II F). Notably, the sum of radioactivity of the 5' cleavage product and the input target RNA were not constant over time, and the 5' cleavage products did not accumulate. Presumably, the cleavage products, once released from the siRNA-endonuclease complex, are rapidly degraded due to the lack of either of the poly(A) tail of the 5'-cap.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-29 (Canceled).

30. (Currently amended) A method for reducing the level of a target mRNA in a cell in vitro comprising contacting a cell with a double stranded RNAi ~~agent~~ molecule which mediates a reduction in the level of a target mRNA, wherein said ~~agent~~ molecule consists of ~~a single continuous double stranded region~~ two separate RNA strands each independently consisting of 19-23 nucleotides, wherein said separate RNA strands form a single continuous double stranded region and wherein at least one strand of said stabilized RNAi molecule has a 3'-overhang from 1-5 nucleotides, ~~said RNAi agent comprises two separate RNA strands each independently consisting of 19-25 nucleotides~~, wherein one of said strands is complementary to a portion of a target mRNA, and said RNAi ~~agent~~ molecule comprises at least one stabilizing modification at the 5' end or the 3' end of the double stranded RNAi molecule, wherein said stabilizing modification is selected from the group consisting of a sugar modified ribonucleotide and a backbone-modified ribonucleotide containing a phosphothioate group, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a ~~group~~ member selected from the group consisting of H,

OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN, wherein R is C_1-C_6 alkyl, alkenyl, alkynyl, and halo is F, Cl, Br or I.

31. (Canceled)

32. (Currently amended) The method of claim 30, wherein both strands of said double-stranded RNAi ~~agent~~ molecule have a 3'-overhang from 1-3 nucleotides.

33. (Currently amended) The method of claim 30, wherein both strands of said double-stranded RNAi ~~agent~~ molecule have a 3'-overhang of 2 nucleotides.

34. (Previously presented) The method of claim 30, wherein each strand has a length from 20-22 nucleotides.

35. (Currently amended) The method of claim 34, wherein both strands of said double-stranded RNAi ~~agent~~ molecule have a 3'-overhang from 1-5 nucleotides.

36. (Currently amended) The method of claim 34, wherein both strands of said double-stranded RNAi ~~agent~~ molecule have a 3'-overhang from 1-3 nucleotides.

37. (Currently amended) The method of claim 34, wherein both strands of said double-stranded RNAi ~~agent~~ molecule have a 3'-overhang of 2 nucleotides.

38. (Currently amended) The method of claim 30, wherein the double-stranded RNAi ~~agent~~ molecule comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR $_1$, NH_2, NHR, NR_2 or CN, wherein R is C_1-C_6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

39. (Currently Amended) The method of claim 30, wherein the double stranded RNAi ~~agent~~ molecule comprises at least one backbone-modified ribonucleotide containing a phosphothioate group.

40. (Currently amended) The method according to claim ~~31~~ 30, wherein said 3' overhang consists of purine nucleotides.

41. (Currently amended) The method according to claim ~~31~~ 30, wherein pyrimidine nucleotides in said 3' overhang is substituted by modified nucleotide analogues.

42. (Previously presented) The method according to claim 41, wherein said modified nucleotide analogues are selected from the group consisting of uridines or cytodines modified at the 5- position, adenosines and guanosines modified at the 8-position, deaza nucleotides, and O- and N- alkylated nucleotides.

43. (Previously presented) The method according to claim 42, wherein said modified nucleotide analogues are selected from the group consisting of 5-(2-amino)propyl uridine, 5-bromo uridine, 8-bromo guanosine, 7-deaza-adenosine, and N6-methyl adenosine.

44. (Currently Amended) The method according to claim 30, wherein said stabilized RNAi ~~agent~~ molecule has two or more different substitutions.

45. (Currently Amended) The method according to claim 44, wherein said stabilized RNAi ~~agent~~ molecule has at least one 2'-OMe sugar modified ribonucleotide and at least one phosphothioate backbone modified ribonucleotide.

46. (Currently Amended) The method according to claim 44, wherein said stabilized RNAi ~~agent~~ molecule has at least one 2'-F sugar modified ribonucleotide and at least one phosphothioate backbone modified ribonucleotide.

47. (Currently amended) The method according to claim ~~31~~ 30, wherein said 3' overhang is comprised of phosphothioate backbone modified ribonucleotides.

48. (Previously presented) The method according to claim 30, wherein one of said RNA strands is complementary to a target nucleic acid.

49. (New) A method for reducing the level of a target mRNA in a cell in vitro comprising contacting a cell with a double stranded RNAi molecule which is capable of RNA interference and which mediates a reduction in the level of a target mRNA, wherein said RNAi molecule consists of two separate RNA strands each independently consisting of 19-23 nucleotides, wherein one of said strands is complementary to a portion of a target mRNA, and wherein said RNA molecule has only one double stranded region, which is a single continuous double stranded region, and said RNAi molecule comprises at least one stabilizing modification selected from the group consisting of a sugar modified ribonucleotide and a backbone-modified ribonucleotide containing a phosphothioate group, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a group selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN, wherein R is C_1-C_6 alkyl, alkenyl, alkynyl, and halo is F, Cl, Br or I.

50. (New) A method for stabilizing an RNAi molecule which is capable of RNA interference and which mediates a reduction in the level of a target mRNA, comprising making at least one stabilizing modification to an RNAi molecule, wherein said stabilizing modification is selected from the group consisting of a sugar modified ribonucleotide and a backbone-modified ribonucleotide containing a phosphothioate group, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a member selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN, wherein R is C_1-C_6 alkyl, alkenyl, alkynyl, and halo is F, Cl, Br or I, resulting in a stabilized RNAi molecule which reduces the level of a target

mRNA in a cell in vitro by mediating a reduction in the level of a target mRNA, wherein said RNAi molecule consists of two separate RNA strands each independently consisting of 19-25 nucleotides, wherein one of said strands is complementary to a portion of a target mRNA, and wherein said RNA molecule has only one double stranded region, which is a single continuous double stranded region.

51. (New) The method according to claim 50, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a member selected from the group consisting of OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN.

52. (New) The method according to claim 30, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a member selected from the group consisting of OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN.

53. (New) The method according to claim 49, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a member selected from the group consisting of OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN.

Amendments to the Drawings

The attached 26 sheets of drawings includes changes to figures 1-19. These figures have been modified as follows:

Figure 1 (A,B) has been modified to include correct pagination.

Figure 2 has been modified to include correct pagination.

Figure 3A has been modified to include correct pagination.

Figure 3B has been modified to include correct pagination and sequence identifiers.

Figure 4A has been modified to include correct pagination and sequence identifiers.

The orientation of the figure was changed from portrait to landscape to provide sufficient space for the addition of the sequence identifiers.

Figure 4B has been modified to include correct pagination.

Figure 5A has been modified to include correct pagination and sequence identifiers.

The orientation of the figure was changed from portrait to landscape to provide sufficient space for the addition of the sequence identifiers.

Figure 5B has been modified to include correct pagination.

Figure 6A has been modified to include correct pagination.

Figure 6B has been modified to include correct pagination.

Figure 7 has been modified to include correct pagination.

Figure 8 has been modified to include correct pagination and sequence identifiers.

Figure 9 has been modified to include correct pagination.

Figure 10 has been modified to include correct pagination.

Figure 11 has been modified to include correct pagination and sequence identifiers.

The orientation of the figure was changed from portrait to landscape to

provide sufficient space for the addition of the sequence identifiers. Furthermore the figure was enlarged to allow for labeling, resulting in an expansion from 1 page to 3 pages. The individual pages have been labeled Figure 11 Part I, Figure 11 Part II and Figure 11 Part III.

Figure 12 has been modified to include correct pagination and sequence identifiers. The figure was enlarged to allow for labeling, resulting in an expansion from 1 page to 2 pages. The individual pages have been labeled Figure 12 Part I and Figure 12 Part II.

Figure 13 has been modified to include correct pagination and sequence identifiers.

Figure 14 has been modified to include correct pagination and sequence identifiers.

Figure 15 has been modified to include correct pagination and sequence identifiers.

Figure 16 has been modified to include correct pagination and sequence identifiers.

Figure 17 has been modified to include correct pagination.

Figure 18 has been modified to include correct pagination and sequence identifiers.

Figure 19 has been modified to include correct pagination and sequence identifiers.

REMARKS:

In the Office Action dated September 19, 2007, claims 30-48 , in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 30-48 remain in this application, new claims 49-52 have been added to the application and claims 1-29 have been canceled.

Substitute drawings are attached to this paper. The drawings have been amended to include sequence identifiers. This labeling required modifications to the drawings including altering the position on the page and enlarging them. The enlarged drawings then occupied additional pages. As a result, all of the drawing pages had to be renumbered.

The amendments to the specification have been made to properly include the sequence identifiers. Additional amendments include the amended drawing designations for Figure 11 (i.e. Parts I-III) and Figure 12 (i.e. Parts I-II) as indicated above. The additions have been indicated by underlining (<u>underlining</u>). The deletions have been indicated by strikethrough (~~strikethrough~~).

The office action indicates that the language "RNAi agent" is not supported by the priority documents. This language has been replaced with "RNAi molecule" which is used in the priority documents. Regarding the range of 19-25 nucleotides, applicants point out that the present application claims priority from PCT/EP01/13968 filed on November 29, 2001 which discloses a range of 19-25 nucleotides. In view of these amendments and comments, applicants contend that the present application is entitled to the benefit of the prior filed applications and/or the foreign priority date of EPO 00126325.0.

Claims 30-48 were rejected under 35 USC §112, second paragraph. Claim 30 has been amended to clarify that the RNAi molecule consists of two separate strands each independently consisting of 19-25 nucleotides, wherein said separate RNA strands form a single continuous double stranded region. Though the RNA strands form only one continuous double stranded region, they may also form single stranded overhangs. In view of the amendments to the claims, applicants request that this rejection be withdrawn.

Claims 30-48 were rejected under 35 USC §112, first paragraph, as including new matter due to the term "RNAi agent". This term has been deleted from the claims and replaced with "RNAi molecule". In view of the above claim amendments, applicants request that this rejection be withdrawn.

Claims 30, 34, 38, 39, 44, 45, and 48 were rejected under 35 USC §102(b) as anticipated by Agrawal. Agrawal discloses self-complementary hairpin oligonucleotides comprising modified building blocks. Agrawal does not disclose a double stranded RNA molecule consisting of two separate strands. Agrawal teaches an olignucleotide where the sense strand and the antisense strand is connected via a polynucleotide linker. In view of the above amendments and discussion, applicants request that this rejection be withdrawn.

Claims 30, 34, 38, 39, 44, and 48 were rejected under 35 USC §102(b) as anticipated by Tracewell. Tracewell discloses the use of double stranded phosphorothioate oligodeoxynucleotides where each strand has a length of 25 nucleotides. Tracewell does not disclose an RNAi molecule consisting of two separate RNA strands each independently consisting of 19-25 nucleotides, wherein said separate RNA strands form a single continuous double stranded region, wherein at least one strand of said stabilized RNAi molecule has a 3'-overhang from 1-5 nucleotides and wherein the RNAi molecule mediates

a reduction in the level of a target mRNA. Applicants also point out that Tracewell's oligodeoxynucleotide is incapable of RNA interference as Tracewell discloses an oligodeoxynucleotide where each building block is a deoxynucleotide building block. As shown in the present application at page 46, example 3.2.4 and figure 14, complete substitution of one or both siRNA strands according to the present invention, by 2'deoxy residues abolishes RNA interference. Therefore, Tracewell does not disclose a double stranded RNAi molecule which mediates a reduction in the level of a target mRNA. In view of the above amendments, applicants request that this rejection be withdrawn.

Claims 30-48 were rejected under 35 USC §102(b) as anticipated by McSwiggen. McSwiggen has an effective filing date of February 20, 2002. The present application claims priority to PCT/EP01/13968 which has a filing date of November 29, 2001. In view of the above amendments and arguments which overcome the new matter rejection, applicants contend that the present application is entitled to the benefit of their PCT priority date and request that this rejection be withdrawn.

Claims 30-48 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as unpatentable over claims 17, 18, 20-24 and 76-80 of co-pending application no. 10/255,568. Applicants respectfully contend that application no. 10/255,568 does not provide an enabling disclosure regarding stabilizing RNAi molecules. There is no disclosure regarding where the modifications should be in the molecule so that the RNAi mediating activity of the molecule is not affected nor any disclosure regarding what nucleotides are useful for stabilizing the RNAi molecule. Co-pending application no. 10/255,568 generally indicates that the RNA molecules can include non-standard nucleotides including non-naturally occurring nucleotides or deoxyribonucleotides but does

not indicate which non-standard nucleotides are useful for stabilizing the RNAi molecule or even that the molecule can be stabilized in this manner without affecting the activity. In contrast to this, the present claims indicate that sugar modified ribonucleotides and backbone-modified ribonucleotides containing a phosphothioate group, wherein said sugar modified ribonucleotide 2'-OH group is replaced by a member selected from the group consisting of H, OR, R, halo, SH, SR, NH_2, NHR, NR_2 and CN, wherein R is C_1-C_6 alkyl, alkenyl, alkynyl, and halo is F, Cl, Br or I, can be used to stabilize the RNAi molecule. Applicants respectfully contend that the general disclosure in co-pending application no. 10/255,568 does not render the modifications recited in the present claims obvious as there is no guidance in co-pending application no. 10/255,568 regarding what modified nucleotides are useful for stabilizing the molecule or where these modifications should be in the molecule. In addition, co-pending application no. 10/255,568 does not indicate that the overhangs should be stabilized and that these stabilizing modifications will not affect the RNAi mediating activity of the molecule. In view of the lack of guidance in co-pending application no. 10/255,568, applicants contend that the present claims would not have been obvious and request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 30-52 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

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